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A RABBIT STUDY OF GLUCOSAMINE SULFATE IN A MODEL OF OSTEOARTHRITIS

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Objective: To determine if orally administered glucosamine sulfate has structure modifying effects in a lapine model of osteoarthritis.

Method: forty five 6-month-old New Zealand white rabbits were divided into groups: Normal control (C); right medial hemimenisectomy (Moskowitz model) alone (OA); right medial hemimenisectomy and glucosamine sulfate 100mg/kg/d (GS100); right medial meniscectomy 200mg/kg/d (GS200). At 12 weeks, the right stifle (knee) cartilage was examined for anatomic change and enzyme activity.

Results: Gross examination—C had no abnormalities, there were mild surface changed in both GS100 and GS200, OA had moderate to marked surface disruptions. Microscopic examination (Mankin histologic scores)—C 1.0±0.0 SD, GS100 3.7±0.8, GS200 4.9±2.0, OA 9.8±1.7. Immunohistochemical staining for MMP-1 and MMP-3—C none, GS100 modest stain, GS200 less stain, OA moderate to marked stain. Substrate gel analysis for MMP2 and MMP-9—C no enzyme, increased both MMP-2 and MMP-9 with less elevation of MMP-3 in GS200 than GS100 than OA. RT-PCR for MMP-1. MMP-3 and MMP-13—C no increase in promoter, increased promoter in all operated groups without difference between treatment and non treatment groups.

Conclusion: In a lapine model of osteoarthritis, GS po was related to a more normal gross and microscopic appearance of articular cartilage. There was less stainable and demonstrable MMP-3 in the GS treated animals. Although there was no significant change with GS in mRNA level of MMP-3 an effect of GS on MMP-3 cannot be excluded.

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FLOATING SECTION IMMUNOCYTOCHEMISTRY: AN ALTERNATIVE METHOD FOR STAINING ARTICULAR CARTILAGE

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Aim: The aim of this study was to develop a robust and reproducible immunocytochemical technique for staining sections of articular cartilage.

Methods: The use of standard immunocytochemical techniques on sections of articular cartilage is complicated by the physical properties of the tissue. This results in poor adhesion and ultimately loss of tissue during the staining procedure. To circumvent this problem we have developed a procedure which utilizes free-floating sections of articular cartilage. Cryostat sections (8um) were cut and immediately fixed in 4% paraformaldehyde for 15 minutes at room temperature. Prior to staining, the sections were washed in PBS, treated with protease-free chondroitinase ABC (0.25U/ml for 1 hour at 37^{O}C), and then washed once more in PBS. Sections were stained using a standard indirect immunoperoxidase technique (Dako, LSAB2, Carpinteria, CA) with the following modifications. The staining reagents were distributed across a 48-well plate (protein block, primary antibody, wash buffer, secondary reagent, wash buffer, tertiary reagent, wash buffer, substrate) such that the sections could be transferred sequentially through the reagents. Transfer of individual sections was achieved using a modified Gilson pipette tip. Following staining, sections were placed on glass slides, counterstained with Mayer's hemotoxylin and allowed to air dry, prior to mounting.

Results: This method has been used extensively to study aggrecanolysis of intact articular cartilage. A second major utility has been in the detection and localization of novel chondrocyte and/or matrix associated antigens. Finally, the method has been further modified to allow the detection of apoptosis using the Tunel staining method.

Conclusions: The floating section technique circumvents the problem of tissue loss associated with standard immunocytochemical methods and offers a robust and reproducible technique for detecting cartilage-associated antigens.

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EFFICIENT TRANSFECTION OF PRIMARY PORCINE AND HUMAN OSTEOARTHRITIC CHONDROCYTES IN SUSPENSION

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Aim: The aim of this study was to develop in chondrocytes an effective non-viral method of gene delivery that retains the differentiated phenotype and has potential for molecular analyses and gene therapy applications.

Methods: Primary articular chondrocytes were isolated from juvenile pigs and osteoarthritic human cartilage. Freshly isolated chondrocytes were transfected with plasmid DNA encoding a CMV-driven green fluorescent protein (GFP) and then maintained in alginate beads. Modification of the existing protocols using Lipofectamine 2000 included orbital shaking at 100 rpm. GFP expression was examined 24 and 48 hours after transfection. Comparisons were made between freshly isolated chondrocytes that received orbital shaking during the 3.5-hour incubation with DNA/reagent complexes and those that did not. Plasmid DNA labeled with the fluorochrome Cy3 was used to assess the mechanism of increased transfection efficiency with agitation. Label incorporation was examined immediately after the 3.5 hour incubation and 1 to 12 hours after rinsing.

Results: Porcine chondrocytes transfected with shaking achieved a transfection efficiency of 20% ± 1.5%, while those that were not agitated yielded 7% ± 0.3%. Primary human articular chondrocytes, from OA cartilage, yielded similar results. With shaking, human chondrocytes had a transfection efficiency of 2l% ± 0.9% and without shaking 8% ± 1.4%. Results were similar 24 and 48 hours after transfection. After 1 week, the high expression level of GFP had caused considerable cell death. Efforts to determine the duration of gene expression in long term 3D culture are under way using less toxic gene products. Fluorescence microscopy of Cy3-labeled plasmid DNA revealed that shaking increased internalization of the DNA.

Conclusions: We have determined that modifications to existing protocols for liposomal transfection, including agitation with an orbital shaker, greatly increase transfection efficiency in primary porcine and human articular chondrocytes in suspension. This increase in transfection efficiency of human chondrocytes occurred without further optimization of protocols developed with porcine chondrocytes. The transfection efficiency achieved with OA human chondrocytes using this method is higher than any reported for non-viral methods. Furthermore, this method, which allows the use of 3D culture conditions throughout, preserves the chondrocyte phenotype by not requiring monolayer adherence.